RECOMBINANT MUCINS, AND COMPOSITIONS AND METHODS FOR USING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application No. 62/792,660, filed Jan. 15, 2019, the entire disclosure of which is incorporated herein by reference.

GOVERNMENT FUNDING

[0002] This invention was made with government support under grant nos. 1DP2GM119133-01 and 1U54CA210184-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

[0003] The disclosure provided improved glycoproteins, and compositions and methods related to the same.

BACKGROUND OF THE DISCLOSURE

[0004] Protein therapeutic agents represent a large and rapidly growing portion of the pharmaceutical market. Current biologics enable the treatment of a wide variety of human diseases, including cancer, autoimmune disorders, arthritis and infectious diseases. The commercial success of biologics has been a major impetus for the development of improved manufacturing technologies that reliably produce the biological agents on a large scale.

[0005] The majority of all recombinant protein pharmaceuticals are produced in mammalian cells at present. Mammalian cells are preferred over prokaryotic organisms for production of protein therapeutics because eukaryote-specific post-translational modifications are often required for protein functionality and appropriate pharmacokinetics. As an example, monoclonal antibodies, a major class of protein therapeutics, must be post-translationally modified with sugar structures called glycans in a post-translational modification process called glycosylation. Without glycosylation, therapeutic antibodies typically have poor stability and pharmacokinetics in vivo.

[0006] Today, the majority of all recombinant protein pharmaceuticals are produced in the mammalian Chinese Hamster Ovary (CHO) cell line. However, a significant drawback CHO cells for bio-manufacturing is their capacity to generate glycans that are not native to humans. These glycans can produce deleterious immune responses and have been implicated in therapeutic resistance, which remains a significant concern for physicians and patients. The risk of patient immune responses from CHO-derived products has motivated a deeper consideration of the use of human cell lines for manufacturing recombinant protein therapies.

[0007] Suspension adapted human embryonic kidney 293 cells (293-F) have become the most popular host cell line for the production of biological therapeutics with human glycosylation patterns. The 293-F cell system has several desirable features for recombinant protein production, including a fast proliferation rate, a high level of protein production, and ease of transient transfection. Recently, the United States Food and Drug Administration (FDA) has approved several therapeutic agents produced in 293-F cells. However, compared to CHO-cell systems, 293-F cells can exhibit a higher propensity to form large aggregates in suspension, limiting their yield and reliability for bio-manufacturing. While special medium formulations have been developed to reduce cell clumping, aggregation continues to be a challenge for mammalian suspension cell culture, especially at the high cell densities required for fast, high-yield protein production. Exogenous addition of anti-clumping agents also introduces additional molecules that must be purified away from secreted protein products. An alternative strategy would be to genetically engineer production cells to have reduced adhesion, but few approaches have been developed at the current time. Thus there is an ongoing, unmet need for alternative compositions and methods for protein production, and for improved glycoproteins that are suitable for use in a number of diverse applications. The present disclosure is pertinent to this need.

SUMMARY OF THE DISCLOSURE

[0008] The disclosure provides modified mammalian cells that express modified polypeptides that act as mucins, and mammalian cell cultures that comprise such cells. In embodiments, the cells comprise recombinant polypeptides expressed from recombinant polynucleotides introduced into the cells.

[0009] In embodiments, the polypeptides comprise a transmembrane anchor and a segment external to the cells. The segment external to the cells includes repeated amino acid sequences. In embodiments, the repeated amino acid sequences are selected from: KEPAPTTP (SEQ ID NO:1); DAATPAP (SEQ ID NO:2); DAATPAPP (SEQ ID NO: 3); PPASTSAPG (SEQ ID NO:4); PDTRPAPGATAPPAHGVTSA (SEQ ID NO:5); PDTRPAPGATAPPAHGVTAA (SEQ ID NO:6); PDARPAPGATAPPAHGVTAA (SEQ ID NO:7); PDTRPAPGSTAPPAHGVTSA (SEQ ID NO:8), and combinations thereof. The repeated amino acid sequence may be repeated contiguously 10-120 times. In certain embodiments, the repeated amino acid sequence is repeated contiguously 21, 40, 42, 59 or 80 times.

[0010] In embodiments, the cells are modified mammalian cells. In embodiments, the cells are modified human cells, which may be human embryonic kidney cells, which in certain embodiments include human 293-F cells. In embodiments, the modified human cells that express modified mucins are adapted to growth in a suspension culture. In embodiments, the modified cells in the suspension culture exhibit less aggregation relative to a suitable control value. A non-limiting example of a suitable control value is a value obtained from a suspended cell culture comprising cells that do not express the recombinant polypeptide comprising the repeated amino acid sequences. The modified cells may be present in a suspension cell culture that is present in a suspended cell bioreactor.

[0011] In certain embodiments, the modified cells express a second, distinct polypeptide. This is achieved by further modifying the cells such that they comprise an introduced polynucleotide encoding a distinct polypeptide that is different from the polypeptide comprising the repeated amino acid sequences.

[0012] In embodiments, the polypeptides expressed by the modified cells exhibit O-glycans on the segment external to the cells. This segment can comprise one or a combination of Core 2 O-glycan, GlcNAcβ1-6(Galβ1-3)GalNAc and/or the Core 2 derivatives of GlcNAcβ1-6(Galβ1-3)GalNAc at